

**WHAT IS CLAIMED IS:**

1. A polynucleotide encoding a promoter operatively linked to a transcriptional unit, wherein the transcription unit encodes a fusion protein, wherein the fusion protein comprises (1) a viral protein, (2) a protein of interest, and (3) an autoproteolytic peptide, wherein (3) is fused between (1) and (2).
2. A polynucleotide according to Claim 1, wherein the viral protein is obtained from a RNA virus.
3. A polynucleotide according to Claim 2, wherein the RNA virus is a plant RNA virus.
4. A polynucleotide according to Claim 3, wherein the plant RNA virus is a plant single-stranded RNA virus.
5. A polynucleotide according to Claim 4, wherein the plant single-stranded RNA virus is a hordeivirus.
6. A polynucleotide according to Claim 5, wherein the hordeivirus is a barley stripe mosaic virus.
7. A polynucleotide according to Claim 1, wherein the autoproteolytic peptide comprises a 2A autoproteolytic peptide.
8. A polynucleotide according to Claim 1, wherein the autoproteolytic peptide comprises the amino acid sequence depicted by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.
9. A polynucleotide according to Claim 1, wherein the fusion protein comprises no more than one viral protein.
10. A polynucleotide according to Claim 1, wherein the viral protein is  $\gamma$ b.

11. A polynucleotide according to Claim 1, wherein the autoproteolytic peptide is fused to the C-terminus of the viral protein.

5 12. A polynucleotide according to Claim 1, wherein the autoproteolytic peptide is fused to the N-terminus of the viral protein.

13. A polynucleotide according to Claim 1, wherein the viral protein is  $\beta$ b.

10 14. A polynucleotide according to Claim 13, wherein the autoproteolytic peptide is fused to the N-terminus of the viral protein, wherein the protein of interest is fused to the N-terminus of the autoproteolytic peptide.

15 15. A polynucleotide according to Claim 1, wherein the protein of interest is a plant protein.

16. A polynucleotide according to Claim 15, wherein the plant protein is a structural protein, enzyme, or a protein involved with pigmentation.

20 17. A recombinant viral nucleic acid comprising the polynucleotide according to Claim 14.

25 18. A plant or plant cell containing the recombinant viral nucleic acid according to Claim 17.

19. A recombinant virus comprising the recombinant viral nucleic acid according to Claim 17, wherein the recombinant virus is capable of systemic expression of the fusion protein.

30 20. A plant or a plant cell infected with a recombinant virus according to Claim 19.

SUB A3) 21. A polynucleotide according to Claim 1, wherein the fusion protein can be expressed in a plant or a plant cell.

22. A recombinant viral nucleic acid comprising the polynucleotide  
5 according to Claim 1.

23. A recombinant virus comprising the recombinant viral nucleic acid according to Claim 22.

10 24. A plant cell infected with a recombinant virus according to Claim 23.

SUB A4) 25. A plant cell according to Claim 24 wherein the plant cell is a monocot plant cell.

15 26. A plant infected with a recombinant virus according to Claim 23.

SUB A5) 27. A plant according to Claim 26 wherein the plant is a monocot plant.

20 28. A polynucleotide according to Claim 1 wherein the protein of interest is a viral protein.

29 A recombinant viral nucleic acid comprising the polynucleotide according to Claim 28.

25 30. A recombinant virus comprising the recombinant viral nucleic acid according to Claim 29.

31. A viral genome comprising one or more polynucleotide(s), wherein at least one polynucleotide encodes a first promoter operatively linked to a  
30 transcriptional unit, wherein the transcription unit encodes a fusion protein, wherein the fusion protein comprises (1) a first viral protein, (2) a second viral protein, and (3) an autoproteolytic peptide, wherein (3) is fused between (1) and (2), wherein at least

one polynucleotide encodes a second promoter operatively linked to the ORF encoding a protein of interest.

5           32.     A viral genome according to Claim 31 wherein the viral genome is the viral genome of a multipartite RNA plant virus.

          33.     A viral genome according to Claim 32 wherein the viral genome is the viral genome of a hordeivirus.

10           34.     A viral genome according to Claim 33 wherein the hordeivirus is a barley stripe mosaic virus.

          35.     A viral genome according to Claim 34 wherein the first viral protein is  $\gamma$ b.

15           36.     A viral genome according to Claim 34 or 35 wherein the second viral protein is  $\beta$ b.

20           37.     A viral genome according to Claim 31 wherein the autoproteolytic peptide comprises the 2A autoproteolytic peptide of a foot and mouth disease virus.

          38.     A viral genome according to Claim 31 wherein the autoproteolytic peptide comprises the amino acid sequence depicted by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, 25     SEQ ID NO: 8, or SEQ ID NO: 9.

          39.     A viral genome according to Claim 31 wherein the protein of interest and the fusion protein are encoded on separate polynucleotides.

30           40.     A viral genome according to Claim 31 wherein the protein of interest and the fusion protein are capable of expression in a plant cell.

41. A viral genome according to Claim 40 wherein the plant cell is a monocot plant cell.

42. A viral genome according to Claim 40 wherein the protein of interest and the fusion protein are capable of systemic expression in a plant.

43. A viral genome according to Claim 42 wherein the plant is a monocot plant.

44. A viral genome comprising one or more polynucleotide(s), wherein at least one of the polynucleotide(s) encodes a promoter operatively linked to a transcriptional unit, wherein the transcription unit encodes a protein of interest, wherein the gene, or a fragment thereof, encoding a coat protein of the viral genome is deleted or mutated, wherein the coat protein is native to the viral genome.

45. A method of expressing a protein of interest at an increased level, comprising:

(a) introducing a viral genome into a host, wherein the viral genome comprising one or more polynucleotide(s), wherein at least one of the polynucleotide(s) encodes a promoter operatively linked to a transcriptional unit, wherein the transcription unit encodes a protein of interest, wherein the gene, or a fragment thereof, encoding a coat protein of the viral genome is deleted or mutated, wherein the coat protein is native to the viral genome, and

(b) growing the host, whereby the protein of interest is expressed at an increased level when compared to using another viral genome that is identical to the viral genome except that the gene encoding the coat protein of the other viral genome is not mutated.

46. A viral genome comprising one or more polynucleotide(s), wherein at least one of the polynucleotide(s) encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes a fusion protein, wherein the gene, or a fragment thereof, encoding a coat protein of the viral genome is deleted or mutated, wherein the coat protein is native to the viral genome, wherein the fusion

protein comprises (1) a viral protein, (2) a protein of interest, and (3) an autoproteolytic peptide, wherein (3) is fused between (1) and (2).

47. A viral genome comprising at least one duplicated genomic nucleic acid component, wherein the duplicated genomic nucleic acid component encodes a promoter operatively linked to a protein of interest, wherein the protein of interest is non-native to the virus, wherein the promoter is native to the virus.

48. A viral genome according to Claim 47 further comprising one or more additional duplicated genomic nucleic acid component(s), wherein each of the additional duplicated genomic nucleic acid component encodes a promoter operatively linked to a protein of interest, wherein each protein of interest may be non-identical to the protein of interest(s) of the other duplicated genomic nucleic acid component(s), wherein each promoter may be non-identical to the promoter(s) of the other duplicated genomic nucleic acid component(s).

49. A viral genome according to Claim 48 wherein the promoters of all the duplicated genomic nucleic acid components are identical to each other.

50. A viral genome according to Claim 49 wherein the protein of interest of all the duplicated genomic nucleic acid components are identical to each other.

51. A viral genome according to Claim 47 wherein the viral genome is the viral genome of a hordeivirus.

52. A viral genome according to Claim 51 wherein the hordeivirus is a barley stripe mosaic virus.

53. A viral genome according to Claim 51 or 52 wherein the duplicated genomic nucleic acid component is a duplicated RNA $\gamma$ .

54. A viral genome according to Claim 53 wherein the promoter is the promoter of the  $\gamma$ b gene.

55. A viral genome according to Claim 54 wherein the  $\gamma$ a gene, or a fragment of the  $\gamma$ a gene, is deleted.

- 5 ~~56.~~ A method of expressing a protein of interest in a host comprising:
- (a) introducing a viral genome into a host; wherein the viral genome comprises at least one duplicated genomic nucleic acid component, wherein the duplicated genomic nucleic acid component encodes a promoter operatively linked to a protein of interest, wherein the protein of interest is non-native to the virus, wherein
- 10 the promoter is native to the virus; and,
- (b) growing the host;
- whereby a protein of interest is expressed in the host.

57. A method according to Claim 56 wherein the viral genome further
- 15 comprises one or more additional duplicated genomic nucleic acid component(s), wherein each of the additional duplicated genomic nucleic acid component encodes a promoter operatively linked to a protein of interest, wherein each protein of interest may be non-identical to the protein of interest(s) of the other duplicated genomic nucleic acid component(s), wherein each promoter may be non-identical to the
- 20 promoter(s) of the other duplicated genomic nucleic acid component(s).

58. A viral genome comprising at least one duplicated genomic nucleic acid component, wherein the duplicated genomic nucleic acid component encodes a promoter operatively linked to a fusion protein, wherein the protein of interest is non-
- 25 native to the virus, wherein the promoter is native to the virus, wherein the fusion protein comprises (1) a viral protein, (2) a protein of interest, and (3) an autoproteolytic peptide, wherein (3) is fused between (1) and (2).

59. A viral genome comprising at least one duplicated genomic nucleic acid component, wherein the duplicated genomic nucleic acid component encodes a promoter operatively linked to a fusion protein, wherein the protein of interest is non-
- 30 native to the virus, wherein the promoter is native to the virus, wherein the fusion protein comprises (1) a viral protein, (2) a protein of interest, and (3) an

autoproteolytic peptide, wherein (3) is fused between (1) and (2), wherein the gene, or fragment thereof, encoding a coat protein of the viral genome is deleted or mutated, wherein the coat protein is native to the viral genome.

5        ~~60.~~     A polynucleotide encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting nucleotide sequence, wherein (1) is 5' of (2), and (2) is 5' of (3), wherein (3) is substantially homologous to a gene of interest, or fragment thereof.

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61.     A polynucleotide according to Claim 60 wherein the presence of the transcriptional unit in a cell results in a decreased expression of the gene of interest, wherein the gene of interest is encoded in a chromosome of the cell.

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~~62.~~     A method of decreasing the expression of a peptide encoded by a gene of interest in a plant host, comprising:

(a) introducing the viral genome of a first virus into a plant host; wherein the viral genome comprises a polynucleotide encoding a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting nucleotide sequence, wherein (1) is 5' of (2), and (2) is 5' of (3), wherein (3) is substantially homologous to a gene of interest, or fragment thereof; and,

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(b) growing the plant host;

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whereby the gene of interest, in the plant host, is expressed at a lower level when compared to using a second virus that is identical to the first virus except that the second virus lacks the targeting nucleotide sequence.

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~~63.~~     A method for causing a phenotypic or biochemical change in a plant host, comprising:

(a) constructing a recombinant plant virus, wherein each recombinant plant virus comprises one or more polynucleotides, wherein at least one polynucleotide encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting



nucleotide sequence, wherein the targeting nucleotide sequence is in a sense or antisense orientation, wherein (1) is 5' of (2), and (3) is 5' of (3), wherein (3) is substantially homologous to a gene of interest, or fragment thereof;

(b) introducing the recombinant plant virus into a plant host; and,

(c) growing the plant host;

whereby a phenotypic or biochemical change is caused in the plant host.

~~64.~~ A method for correlating a nucleotide sequence of a donor organism with its function comprising:

(a) constructing a recombinant plant viral genome library, wherein each recombinant plant viral genome of the library encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting nucleotide sequence, wherein (3) comprises an insert from a library of donor organism derived cDNA, genomic DNA, or genomic RNA, wherein the insert is in a sense or antisense orientation, wherein (1) is 5' of (2), and (3) is 5' of (3);

(b) expressing each transcriptional unit in a plant host;

(c) detecting one or more phenotypic or biochemical change of the plant host resulting from the expression of the transcriptional unit;

(d) determining the nucleotide sequence of the insert; and

(e) correlating the nucleotide sequence of the insert with the phenotypic or biochemical change;

whereby a nucleotide sequence of a donor organism is correlated with its function.

~~65.~~ A viral genome comprising one or more polynucleotide(s), wherein at least one of the polynucleotide(s) encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting nucleotide sequence, wherein (1) is 5' of (2), and (2) is 5' of (3), wherein (3) is substantially homologous to a gene of interest, or fragment thereof, wherein the gene, or a fragment thereof, encoding a coat protein of the viral genome is deleted or mutated, wherein the coat protein is native to the viral genome.

~~66.~~ A viral genome comprising at least one duplicated genomic nucleic acid component, wherein the duplicated genomic nucleic acid component encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting nucleotide sequence, wherein (1) is 5' of (2), and (2) is 5' of (3), wherein (3) is substantially homologous to a gene of interest, wherein the promoter is native to the viral genome.

~~67.~~ A viral genome comprising at least one duplicated genomic nucleic acid component, wherein the duplicated genomic nucleic acid component encodes a promoter operatively linked to a transcriptional unit, wherein the transcriptional unit encodes (1) a viral protein, (2) a stop codon, and (3) a targeting nucleotide sequence, wherein (1) is 5' of (2), and (2) is 5' of (3), wherein (3) is substantially homologous to a gene of interest, wherein the promoter is native to the viral genome, wherein a gene, or a fragment thereof, encoding a coat protein of the viral genome is deleted or mutated, wherein the coat protein is native to the viral genome.

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